εBP, a β-Galactoside-Binding Animal Lectin, Recognizes IgE Receptor (FcεRI) and Activates Mast Cells†

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ABSTRACT: IgE-binding protein (εBP) was originally identified in rat basophilic leukemia (RBL) cells by virtue of its affinity for IgE. εBP is now known to be a β-galactoside-binding lectin containing an S-type carbohydrate recognition domain. It is identical to a macrophage surface antigen, Mac-2, and lectins designated as CBP35, L-34, and RL-29, for which various functions have been suggested. Studies from other groups as well as ours have indicated that εBP is secreted by cells such as macrophages and is present in extracellular fluids. We demonstrated previously that binding sites for εBP are present on the surface of RBL cells. In this report, we show that εBP binds to a small number of glycoprotein species on the surface of RBL cells. Significantly, one of these glycoproteins is the high-affinity IgE receptor (FcεRI). Preliminary studies showed that εBP causes mediator release from RBL cells, possibly through cross-linking of FcεRI. The results suggest a function of εBP as an activator of mast cells.

IgE receptor (FcεRI)[1] present on mast cells and basophils is a key component responsible for IgE-mediated allergic reactions. Multivalent allergens bind to the receptor-bound IgE and cause cross-linking of the receptor, resulting in mediator release from mast cells and basophils (Metzger et al., 1986). FcεRI is a glycoprotein with a tetrameric structure: one IgE-binding α-subunit, one β-subunit, and two γ-subunits (Metzger, 1991; Ravetch & Kinet, 1991). The primary structure of this receptor has been elucidated. However, the structure and functional significance of the oligosaccharides linked to the protein core are largely unknown. It is likely that the function of oligosaccharide components of glycoproteins is directly related to their recognition by some of the endogenous lectins. In this paper we report that FcεRI is indeed recognized by an animal lectin, the IgE-binding protein (εBP).

εBP is an M_r 31 000 lectin composed of two domains with the amino-terminal domain containing a highly conserved repetitive sequence and the carboxyl-terminal half consisting of an S-type carbohydrate recognition domain (Liu, 1990; Drickamer, 1988). We designated it as IgE-binding protein because it was originally identified by its ability to bind IgE (Liu et al., 1985; Albrandt et al., 1987). However, IgE binding is now considered as only one of εPB's many functions. This protein has been designated by different names, CBP35 (Jia & Wang, 1988; Moutsatsos et al., 1987; Laing et al., 1989), L-34 (Raz et al., 1989), Mac-2 (Cherayil et al., 1989), and RL-29/HL-29 (Sparrow et al., 1987; Leffler & Barondes, 1986), and described with various possible functions such as growth regulation, a role in tumor metastasis, and as a macrophage surface differentiation marker. εBP is also identical to a major non-integrin laminin-binding protein (Woo et al., 1990), suggesting a possible function of this protein in cell adhesion to basement membranes.

Previously we demonstrated that εBP is present on the surface of mast cells bound to then unidentified glycoconjugates on the cell surface (Frigeri & Liu, 1992). Furthermore, various mast cell and macrophage lines were shown to contain εBP-binding sites (Frigeri & Liu, 1992). To understand further the nature of εBP-binding sites on mast cells and how εBP may affect the function of these cells, we analyzed the glycoprotein(s) that εBP binds to on the surface of RBL cells.

MATERIALS AND METHODS

Cells and Reagents. Rat basophilic leukemia (RBL-2H3) cells were obtained from Dr. Baird (Department of Chemistry, Cornell University, Ithaca, NY) and were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 20% heat-inactivated fetal bovine serum, 2 mM glutamine, penicillin, and streptomycin. Rat peritoneal mast cells were obtained by peritoneal lavage of Lewis rats (The Scripps Research Institute rodent colony, La Jolla, CA) as previously described (Bach et al., 1971).

Preparation of recombinant rat εBP (rεBP) (Frigeri et al., 1990) and mouse monoclonal anti-dinitrophenol (DNP) IgE (Liu et al., 1980) was described previously. Monoclonal antibodies directed against the α-subunit of the high-affinity IgE receptor (mAb TW) or the β-subunit (mAb NB), both of the IgG_1 subclass, were a generous gift of Drs. B. Baird and D. Holowka (Department of Chemistry, Cornell University). Rabbit anti-mouse IgG (RAMG) was from Zymed Laboratories Inc., San Francisco, CA. Unless otherwise specified, all common reagents used were obtained from Sigma (St. Louis, MO). Proteins were coupled to CNBr-activated Sepharose 4B (Pharmacia, Piscataway, NJ) following the manufacturer's instructions and using the following ratios of protein to CNBr-activated Sepharose 4B (dry weight): rεBP–Sepharose 4B, 1 mg/0.4 g; anti-DNP IgE–Sepharose 4B, 10 mg/1 g; RAMG–Sepharose 4B, 10 mg/1 g; mAb TW–Sepharose 4B, 10 mg/1 g.

Cell-Surface Iodination and Preparation of Cell Lysates. Mast cell-surface proteins were iodinated using lactoperoxidase as described previously (Morrison, 1974). Briefly, cells were harvested in phosphate-buffered saline (PBS) containing 5

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[1] Abbreviations: εBP, IgE-binding protein; rεBP, recombinant rat εBP; RBL, rat basophilic leukemia; Mac-2, macrophage surface antigen of M_r 32 000; FcεRI, high-affinity IgE receptor; DTSSP, 3,3′-dithiobis-(sulfosuccinimidyl propionate); RAMG, rabbit anti-mouse IgG.

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mM EDTA, washed once in PBS/10 μM potassium iodide, and resuspended in the same buffer at 2×10^7 cells/mL. For each milliliter of cell suspension, the following reagents were added: 2.34 units of lactoperoxidase (78 units/mg); 500 μCi of $Na^{125}I$ (Amersham, Arlington Heights, IL); 10 μL of 0.03% H_2O_2 in PBS. After 1 min at 25 °C, the same amount of H_2O_2 was added, and the mixture was incubated at 25 °C for an additional minute. Thereafter, 10 mL of DMEM containing 20% calf serum (DMEM–CS) was added. Cells were centrifuged for 10 min at 800*g*, 4 °C, and then washed twice in DMEM–CS and PBS sequentially, by centrifugation as above. Cells were lysed (1×10^7 cells/mL) by using a buffer containing protease inhibitors as previously described (Liu & Orida, 1984). Labeling efficiency was in the range of 2×10^6 to 8.3×10^6 cpm/1×10^6 cells.

Labeling of Cell-Surface Glycoproteins with Tritium. Labeling of cell-surface glycoproteins with tritium was performed using galactose oxidase according to a published procedure (Gahmberg & Hakomori, 1973). RBL cells (3.25×10^7) were harvested as described above, resuspended in PBS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 units of galactose oxidase (250 units/mL), and incubated for 30 min at 37 °C. After two washes in cold PBS, cells were resuspended in 1 mL of PBS/10 mM HEPES, pH 7.0, followed by the addition of 1 mCi of Na^3HB_4 (15 mCi/mL). After a 30-min incubation at 25 °C, the cells were washed extensively with cold PBS and lysed as described above. Labeling efficiency was 2.8×10^4 cpm/1×10^6 cells. The cell lysate (2.5×10^7 cell equiv) was subjected to affinity purification using rεBP–Sepharose 4B as described in the following section.

Affinity Purification of RBL Cell-Surface Glycoproteins Recognized by εBP. Lysate (120 μL of 1×10^7 cells/mL) from surface-iodinated cells was mixed with immobilized rεBP (50 μL of beads) in the presence or absence of 50 mM lactose for 3 h at 4 °C. The beads were washed 3 times with 1.3 mL of 1% Triton X-100/10 mM Tris-HCl (pH 7.5)/5 mM EDTA/150 mM NaCl (TEN-1% Triton buffer) and twice with 1.3 mL of 62.5 mM Tris-HCl, pH 6.8. The bound protein was eluted from the beads with 75 μL of SDS–PAGE sample buffer [62.5 mM Tris-HCl (pH 6.8)/2% SDS/10% glycerol/5% 2-mercaptoethanol] in a boiling water bath for 3 min, or with three times of 100 μL of 50 mM lactose containing 1% Triton X-100.

Affinity Purification of the High-Affinity IgE Receptor (FcεRI). (A) From Cell Lysates: One Cycle. Lysates from cell-surface-iodinated RBL cells or rat peritoneal mast cells were mixed with either IgE–Sepharose 4B or mAb TW–Sepharose 4B (50 μL of beads each) for 3 h at 4 °C. After being washed (performed as described above), the bound protein was eluted by boiling in SDS–PAGE sample buffer.

(B) From Cell Lysates: Two Cycles. The protein bound to IgE–Sepharose 4B described in part A above was eluted with 0.5 N acetic acid/1% Triton X-100 (3×100 μL) and immediately neutralized with 2 M Tris-HCl, pH 8.2 (3×37.5 μL). The eluted protein was mixed with IgE–Sepharose 4B or rεBP–Sepharose 4B (50 μL), washed as described above, and eluted with 75 μL of SDS–PAGE sample buffer.

(C) From the Mixture of Proteins Purified by εBP–Sepharose 4B. The protein from lysates of surface-iodinated cells (1.5×10^7) that was bound to rεBP–Sepharose 4B and eluted with 0.1 M lactose as described above was mixed with IgE–Sepharose 4B or mAb TW–Sepharose 4B. The bound protein was eluted with SDS–PAGE sample buffer.

Alternatively, the εBP-purified proteins (from 1.5×10^7 cells) were incubated (1 h, 25 °C) with 5 μg of monoclonal antibody TW or NB. Anti-DNP IgG_1 was used as a control for nonspecific binding. After incubation, RAMG–Sepharose 4B was added, and the mixture was incubated further for 4 h at 4 °C. The beads were washed as described earlier, and the bound protein was eluted with SDS–PAGE sample buffer.

Cross-Linking of ^{125}I-rεBP to FcεRI with 3,3′-Dithiobis(sulfosuccinimidyl propionate) (DTSSP). Cross-linking of rεBP with RBL cells was performed using a water-soluble, membrane-impermeable reagent, DTSSP, following published procedures (Lee & Conrad, 1985). Recombinant εBP was iodinated by the chloramine-T method (McConahey & Dixon, 1966) using 10 μg of protein and 0.5 mCi of ^{125}I. RBL cells (6×10^7) were harvested, washed once in PBS, resuspended in 6 mL of DMEM/10 mM HEPES, pH 7.5, and equally divided into three fractions. To fraction 1 was added 60 pmol of ^{125}I-rεBP (specific activity 1.68×10^{15} cpm/mmol) followed by 4 μL of 0.5 M DTSSP in DMSO (final concentration of DTSSP, 2 mM); in fraction 2, cells were exposed to ^{125}I-rεBP without DTSSP; in fraction 3, cells were exposed to ^{125}I-rεBP in the presence of 50 mM lactose. The mixtures were incubated for 30 min at 4 °C, and then the cells were washed 3 times with 5 mL of 25 mM Tris–HCl in PBS, pH 7.5, and lysed in TEN–1% Triton buffer as described before. Cell lysates were mixed with mAb TW–Sepharose 4B (50 μL of beads) or anti-DNP IgG_1–Sepharose 4B (as a control) for 3 h at 4 °C. The bound protein was eluted with SDS–PAGE sample buffer and detected by autoradiography after SDS–PAGE separation.

Determination of Serotonin Release from RBL Cells. Cells were harvested in PBS/5 mM EDTA, washed once in DMEM containing 10% FBS, resuspended at 3×10^6 cells/mL, and incubated with 5–[^{3}H]hydroxytryptamine binoxalate (10 μCi/mL final concentration) for 1 h at 37 °C. Cells were washed twice in DMEM containing 0.5% BSA. They were then aliquoted into separate centrifuge tubes containing appropriate concentrations of rεBP, with or without saccharides used as inhibitors of εBP. As a control, appropriate dilutions of PBS/10% glycerol (the buffer in which εBP is dissolved) were used instead of εBP. The final concentration of glycerol used was maximally 0.8%. After 45 min of incubation at 24 °C on a nutator, cells were centrifuged (800*g*, 5 min at 24 °C), washed once in DMEM/0.5% BSA, and diluted in the same medium to 4×10^5 cells/mL. Cells (0.5 mL) were transferred to a 24-well tissue culture plate (Falcon, Lincoln Park, NJ) and incubated at 37 °C for 2 h. Afterwards, the supernatant of the individual wells was transferred to centrifuge tubes and centrifuged (800*g*, 5 min at 4 °C). The cells remaining adherent to the plate were lysed in 0.3 mL of PBS/1% Triton X-100 at 37 °C for 30 min. Radioactivity in the supernatants and lysates was counted in an automatic liquid scintillation counter. The percentage of [^{3}H]serotonin released was calculated by dividing the total cpm recovered in the supernatant (released) over the total radioactivity taken up by the cells (supernatant + lysate). Five replicates per point were used, and statistical significance was estimated by ANOVA and multiple-range analysis.

SDS–PAGE and Autoradiography. Proteins were separated by SDS–PAGE according to Laemmli (1970), and gels were dried for 1 h at 65 °C under reduced pressure. Radiolabeled bands were visualized by autoradiography using Kodak X-Omat film in the presence of an enhancing screen. ^{3}H-Labeled glycoproteins were detected by fluorography after treating the gel with EN^{3}HANCE (New England Nuclear, Boston, MA).

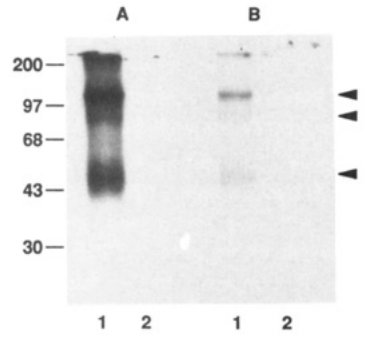

FIGURE 1: Autoradiography of SDS–PAGE analysis of glycoproteins on the surface of RBL cells recognized by ϵBP. Lysate from surface-iodinated RBL cells (1.2×10^6 cell equiv) was mixed with rϵBP–Sepharose 4B in the absence (panels A and B, lane 1) or presence (panels A and B, lane 2) of 50 mM lactose. The bound protein was eluted with SDS–PAGE sample buffer (panel A, lanes 1 and 2) or *0.1 M* lactose/1% Triton X-100 (panel B, lanes 1 and 2) followed by SDS–PAGE (10% polyacrylamide) and autoradiography (72 h). Positions of molecular weight markers ($\times 10^{-3}$) are indicated on the left margin. Positions of proteins of M_r 50 000, 93 000, and 150 000 are indicated by arrows on the right margin.

RESULTS

ϵBP Recognizes a Small Number of Glycoproteins on the Surface of RBL Cells. Previously, we determined that there are approximately 9×10^4 ϵBP-binding sites per cell on the surface of RBL cells (Frigeri & Liu, 1992). In order to elucidate the nature of cell-surface ϵBP-binding glycoproteins, we labeled the RBL cells with ^{125}I and affinity-purified the ligands from the cell lysate with rϵBP immobilized on Sepharose 4B. The bound protein was eluted with either SDS or 0.1 M lactose and analyzed by SDS–PAGE. As shown in Figure 1, only two major proteins of M_r 50 000 and 150 000 and a minor one of M_r 93 000 (indicated by arrows) were detected in both cases (panels A and B, lane 1). The top band ($M_r > 220\,000$) probably represents high molecular weight aggregates that did not enter the gel because it was also observed occasionally when RBL lysate was immunoprecipitated with IgE or the anti-FcϵRI mAb TW. The fact that the three proteins detected could be eluted with lactose indicates that ϵBP, a β-galactose-binding lectin, binds to these proteins via lectin–carbohydrate interaction. The specificity of the interaction was supported further by lack of detection of any protein when the lysate was mixed with rϵBP–Sepharose 4B in the presence of lactose (panels A and B, lane 2). It was noted that the recovery of ϵBP-reactive proteins was less when the lactose was used as the eluent as compared to SDS (Figure 1, panel A vs panel B). This probably reflects the relative inefficiency of the monovalent lactose to compete with the multivalent, higher affinity interaction between ϵBP and the glycoprotein ligands.

As an alternative approach to demonstrating the specificity of surface glycoprotein recognition by ϵBP, we also surface-labeled the RBL cells with galactose oxidase followed by reduction with Na^3HB_4. Analysis of the whole cell lysate by SDS–PAGE confirmed that many glycoproteins are labeled using this procedure (Figure 2, lane 1). However, only one band of approximately M_r 50 000 was affinity-purified by rϵBP–Sepharose 4B (Figure 2, lane 2).

One of the ϵBP-Reactive Glycoproteins Is the High-Affinity IgE Receptor. The α-subunit of the high-affinity IgE receptor (FcϵRIα) is of M_r 50 000, highly glycosylated (Metzger et al., 1986), and recognized by various plant lectins (Helm & Froese, 1981). We thus suspected that the M_r 50 000 protein detected by ϵBP might be FcϵRIα. To test this hypothesis,

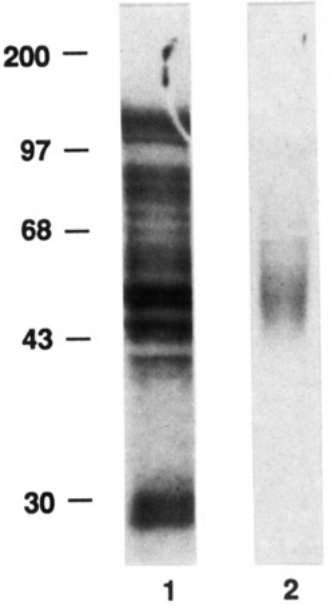

FIGURE 2: SDS–PAGE analysis of RBL cell-surface glycoproteins labeled with tritium. The lysate (6×10^7 cell equiv) from RBL cells labeled with tritium was mixed with rϵBP–Sepharose 4B. The bound protein was then eluted with SDS sample buffer and analyzed. The total cell lysate (from 6×10^6 cell equiv, lane 1) and the ϵBP-binding glycoproteins (lane 2) were analyzed by SDS–PAGE (10% polyacrylamide), followed by fluorography. Exposure time was 14 days.

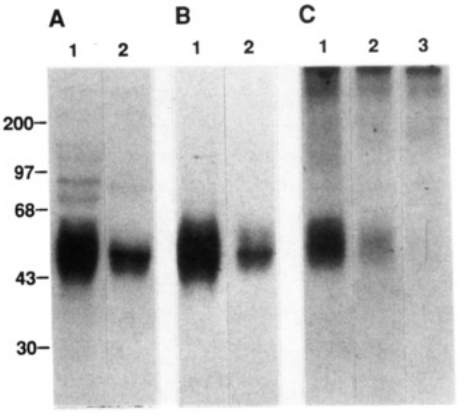

FIGURE 3: The M_r 50 000 ϵBP-reactive glycoprotein on the RBL cell surface is the IgE receptor. *Panel A*: Lysate from surface-iodinated RBL cells (5×10^6 cells) was divided into two equal fractions. One fraction was adsorbed with IgE–Sepharose 4B, washed, and eluted with SDS–PAGE sample buffer (lane 1). The second fraction was adsorbed with anti-FcϵRIα mAb TW–Sepharose 4B and treated as above (lane 2). *Panel B*: Lysate from surface-iodinated RBL cells (1.5×10^7 cells) was mixed with rϵBP–Sepharose 4B, and the bound protein was eluted with 50 mM lactose/1% Triton X-100 and adsorbed with IgE–Sepharose 4B (lane 1) or mAb TW–Sepharose 4B (lane 2) followed by elution with SDS–PAGE sample buffer. *Panel C*: Lysate from surface–iodinated RBL cells (4.5×10^7 cells) was mixed with rϵBP–Sepharose 4B. The bound protein was eluted with lactose, divided into three equal fractions, and subsequently mixed with anti-FcϵRIα mAb TW (lane 1), anti-FcϵRIβ mAb NB (lane 2), or anti DNP-IgG_1 mAb (lane 3). The immune complexes were bound to rabbit anti-mouse IgG (RAMG) coupled to Sepharose 4B, eluted with SDS–PAGE sample buffer, and analyzed as described above. All purified materials were analyzed by SDS–PAGE (10% polyacrylamide) followed by autoradiography (pane A, 68 h; panel B, 44 h; panel C, 85 h).

the proteins bound to rϵBP–Sepharose 4B and eluted with lactose were subjected to affinity purification with IgE or anti-FcϵRIα mAb TW. As shown in Figure 3B, the M_r 50 000 ϵBP-reactive protein indeed bound both IgE (lane 1) and mAb TW (lane 2). This protein has mobility on the SDS–PAGE

gel that is identical to FcεRIα purified directly from ^{125}I-labeled RBL cells by either IgE (panel A, lane 1) or mAB TW (panel A, lane 2).

FcεRI consists of a β-subunit and two γ-subunits that are noncovalently associated with the α-subunit (Metzger, 1991). It has been shown previously that all subunits remain associated when cells are solubilized by detergents under appropriate conditions (Kinet et al., 1985) and antibody to the β-subunit copurifies the α-subunit. To further confirm that εBP binds FcεRI, we used another mAb, NB, directed to the β-subunit of the receptor. Proteins purified by εBP were reacted with mouse antibodies: (i) mAb TW; (ii) mAb NB; or (iii) an anti-DNP mAb used as a control for nonspecific binding. The immune complexes were subsequently bound to rabbit anti-mouse IgG (RAMG) conjugated to Sepharose 4B. Figure 3 (panel C) shows that the M_r 50 000 protein was purified by both anti-FcεRI mAbs TW and NB (lanes 1 and 2, respectively) whereas it was not bound to control mAb (lane 3). It is to be noted that because the β-subunit is generally not labeled when RBL cells are subjected to surface iodination, only the α-subunit and not the β-subunit was detectable on the autoradiographs of SDS–PAGE gel. The fact that considerably less protein bound to mAb NB as compared to mAb TW most likely reflects substantial dissociation of the α- and β-subunits of the receptor under our experimental conditions.

^{125}I-εBP Can Be Conjugated to IgE Receptor on RBL Cells by a Chemical Cross-Linker. The results described above clearly demonstrated that one of the ligands for εBP on the surface of RBL cells is the IgE receptor. However, in all of the above experiments, the binding of the ligands to εBP was shown with solubilized cell membranes, and we did not know whether the lectin could recognize the native form of the receptor on surfaces of intact cells. To test this, we cross-linked radiolabeled εBP to RBL cells with a membrane-impermeable, thiol-cleavable cross-linker, dithiobis(sulfosuccinimidyl propionate) (DTSSP), and subsequently detected radiolabeled εBP that copurified with FcεRI. Thus, ^{125}I-rεBP was reacted with RBL cells (i) in the presence of the cross-linker, (ii) in the absence of the cross-linker, and (iii) in the absence of the cross-linker and in the presence of lactose. Cells were lysed, and the lysate was subjected to immunoprecipitation with mAb TW or control IgG1 mAb. The purified material was then analyzed by SDS–PAGE under reducing conditions that cleaves the disulfide bond of the cross-linker and releases the bound ^{125}I-εBP which was subsequently detected by autoradiography. We found that even without the use of the cross-linker, εBP (M_r 30 000) is copurified with FcεRI (Figure 4, lane 3). However, more εBP was recovered when the cross-linker was used (lane 1). Furthermore, ^{125}I-εBP was not detected from RBL cells treated with ^{125}I-εBP in the presence of lactose (lane 5) nor from immunoprecipitation with control mAb (lanes 2, 4, and 6). The results indicated that εBP binds to FcεRI on intact cells in a fashion that remains stable after the membrane is solubilized. However, the FcεRI–εBP binding appears to be reversible under the experimental conditions, as the use of the cross-linker resulted in a significantly higher amount of detectable ^{125}I-εBP linked to FcεRI.

εBP Recognizes the IgE Receptor on Rat Peritoneal Mast Cells. Because RBL cells are derived from neoplastic leukemia cells, it is possible that recognition of FcεRI on these cells by εBP is a result of the presence of oligosaccharide structures unique to these cells. Therefore, it was important to confirm our findings using normal mast cells. For this purpose, we isolated ^{125}I-labeled FcεRI from both RBL cells and rat peritoneal mast cells and evaluated the binding of the purified receptor to εBP. In both cases, binding of the receptor to IgE–Sepharose 4B was included for comparison. As shown in Figure 5, the amount of ^{125}I-labeled FcεRI bound to εBP–Sepharose 4B (panels A and B, lane 2) was comparable to that bound to IgE–Sepharose 4B (panels A and B, lane 1) for both RBL cells and rat peritoneal mast cells. On the basis of the amount of radioactivity bound to the adsorbents in two separate experiments, it was calculated that ^{125}I-FcεRI that bound to εBP was 68% and 78% of that bound to IgE, for RBL cells and rat peritoneal mast cells, respectively. Therefore, we conclude that recognition of FcεRI by εBP is not unique to transformed mast cells and that a substantial proportion of the FcεRI protein molecules from both transformed and nontransformed mast cells are recognized by εBP.

εBP Induces Serotonin Secretion from RBL Cells. The εBP, like other lectins, is known to function at least bivalently, since it causes hemagglutination of red blood cells (Frigeri et al., 1990). An obvious question was whether the binding of

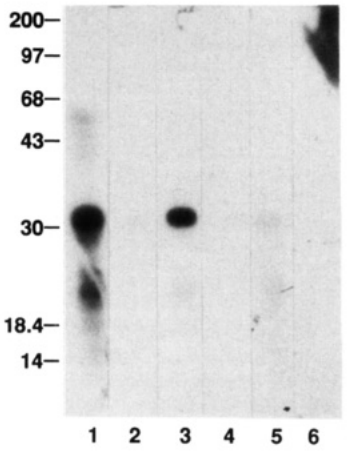

FIGURE 4: Cross-linking of ^{125}I-rεBP to the IgE receptor of RBL cells by DTSSP. RBL cells were divided into three fractions, and each was treated with an equal amount of ^{125}I-rεBP (60 pmol) followed by 2 mM DTSSP (lanes 1 and 2), buffer alone (lanes 3 and 4), or 50 mM lactose (lanes 5 and 6). The cell lysates from the treated cells were subjected to immunoprecipitation with mAb TW–Sepharose 4B (lanes 1, 3, and 5) or with mAb anti-DNP IgG_1–Sepharose 4B (lanes 2, 4, and 6) as control.

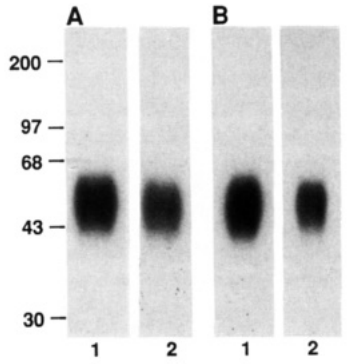

FIGURE 5: εBP binds purified IgE receptor from peritoneal mast cells. *Panel A*: Lysate of ^{125}I-labeled RBL cells (3.5×10^7 cells) was adsorbed with IgE–Sepharose 4B, and the bound protein was eluted with 0.5 N acetic acid/1% Triton X-100 and neutralized as described under Materials and Methods. The affinity-purified protein was then bound to IgE–Sepharose 4B (lane 1) or rεBP–Sepharose 4B (lane 2) and subsequently eluted with SDS. *Panel B*: The same protocol as above was used to purify the IgE receptor from ^{125}I-labeled rat peritoneal mast cells (7.2×10^6 cells). Lane 1, IgE–Sepharose 4B eluate; lane 2, rεBP–Sepharose 4B eluate. The protein was analyzed by SDS–PAGE (10% polyacrylamide) followed by autoradiography. Exposure time was 30 h.

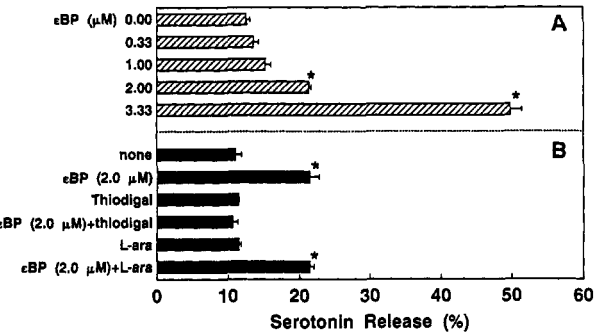

FIGURE 6: εBP-dependent secretion of [^{3}H]serotonin by RBL cells. *Panel B*: RBL cells labeled with [^{3}H]serotonin were incubated with varying amounts of rεBP and, after washing, plated on culture plates. After incubation, the radioactivity in the supernatants and the cells was determined. The percentage of serotonin release was calculated as described under Materials and Methods. rεBP caused significant serotonin release ($P < 0.01$) at 2 and 3 μM concentrations. *Panel B*: [^{3}H]Serotonin-labeled RBL cells were incubated with rεBP in the presence or absence of saccharides and treated as in panel A to determine the percentage of serotonin release. Thiodigalactoside (thiodigal) but not L-arabinose (L-ara) significantly inhibited the action of εBP ($P < 0.01$). The points are the mean ± SD of five determinations from a representative experiment. Experiments were repeated 3 times.

εBP to the FcεRI on mast cells would cause the secretion of mediators by cross-linking the receptors. When rεBP was added to a suspension of RBL cells or to cells attached to plastic wells, no serotonin release was observed (data not shown). However, when the cells were preincubated with appropriate amounts of rεBP and then allowed to adhere to the plate, a lectin-dependent mediator release was consistently observed (Figure 6). Under the experimental conditions described, there was less than 12% release of [^{3}H]serotonin from untreated RBL cells. For RBL cells treated with rεBP at 2 μM (60 μg/mL), over 21% of [^{3}H]serotonin release was noted. A more pronounced release (50%) was observed for cells treated with rεBP at 3 μM (90 μg/mL) (Figure 6A). The effect of εBP was completely inhibited by thiodigalactoside, a carbohydrate that binds specifically to the lectin (Figure 6B). L-Arabinose, which does not bind εBP, failed to inhibit the serotonin release (Figure 6B). For this experiment, thiodigalactoside was used instead of lactose because of its higher affinity (2.5-fold) for εBP (Leffler & Barondes, 1986; Frigeri et al., 1990). Thus, a lower concentration of saccharide could be used, reducing the possible detrimental effect of high osmolarity to the cells.

DISCUSSION

From data presented herein, we conclude that εBP binds to the high-affinity IgE receptor (FcεRI) on mast cells. This conclusion is based on (i) identification of one of the εBP-reactive glycoproteins on the surface of RBL cells as FcεRI and (ii) demonstration that purified FcεRI binds to εBP. The binding of εBP to FcεRI on intact cells was also shown. Furthermore, FcεRI from both a tumor cell line, RBL, and normal peritoneal mast cells was recognized by εBP. On the basis of our knowledge of εBP and the fact that the interaction of FcεRI with εBP is inhibitable by lactose, the most likely explanation for our results is that εBP recognizes the β-galactoside structure on the α-subunit of FcεRI.

The finding that only a small number of glycoproteins on the surface of RBL cells are recognized by εBP indicates the selectivity of the recognition process. Such selectivity has been well demonstrated for the various members of the S-type lectin family (Leffler & Barondes, 1986; Sparrow et al., 1987). Recently, Rosenberg et al. reported the isolation of two glycoproteins from intestinal epithelioma cell lines that bind to Mac-2 (identical to εBP) (Rosenberg et al., 1991). The results are consistent with the restricted recognition of glycoproteins by this type of lectin. On the other hand, it is also clear that εBP, initially identified by its IgE-binding activity, also binds a number of other glycoproteins. εBP and related lectins may thus be viewed to have possibly wide-ranging functions by recognizing various cells and tissues.

In repeated experiments, we noted that not all purified FcεRI bound to εBP. Although the results could be due to partial denaturation of the purified receptor resulting in the loss of recognition by εBP, a possible explanation is the microheterogeneity in glycosylation of FcεRI. More specifically, a number of glycoforms of FcεRI may exist varying in recognition by εBP. Previously, we have shown the existence of IgE glycoforms that are differentially recognized by εBP (Robertson et al., 1990; Robertson & Liu, 1991). In that case, experimental data suggested that these various glycoforms differ in the degree of sialylation. Similar heterogeneity in sialylation may exist for FcεRI. It is possible that expression of various FcεRI glycoforms is correlated with the differentiation of the mast cells and functional heterogeneity of FcεRI.

The finding that εBP binds both IgE and the IgE receptor supports a role for this protein in immediate hypersensitivity. εBP is expressed constitutively by mast cells as well as many other cell types, and it is also secreted by certain cell types. εBP/Mac-2 was shown to be secreted by macrophages from thioglycolate-treated mice (Cherayil et al., 1989). We have also detected εBP in peritoneal exudates in mice treated with thioglycolate (L. G. Frigeri, unpublished results). Therefore, εBP from various sources can bind FcεRI on the surface of mast cells. Since εBP functions at least bivalently (Frigeri et al., 1990), it has the potential to cross-link two FcεRI proteins, directly or via IgE, or conjugate one receptor protein to other cell-surface glycoproteins. In this way, εBP may activate mast cells or otherwise influence the FcεRI-mediated activation of mast cells, especially since it is well established that receptor aggregation signals mast cell activation (Ishizaka & Ishizaka, 1978; Metzger, 1978; Metzger et al., 1986).

The degranulation study (Figure 6) supports the hypothesis that εBP can activate mast cells. However, the mechanism of this activation is not clear. It is interesting that cells treated with εBP need to be allowed to adhere to the culture plates for the serotonin release to occur. No release above the background levels has been observed by treating RBL cells with εBP in solution. In addition, the activation of RBL cells by εBP was observed at relatively high εBP concentrations. Previously, we found that at these concentrations of εBP, there is a positive cooperative binding of εBP to IgE (Hsu et al., 1992), and we have proposed a model that εBP self-associates to form dimers, or higher oligomers, at these concentrations. The described activity of εBP is thus correlated with the potential of this lectin to cross-link cell-surface glycoproteins such as FcεRI. The adhesion of εBP-treated cells to plastics may further facilitate aggregation of the receptors. It is not possible to state whether the high concentrations of εBP used in this study can be achieved physiologically. It is to be noted that L-29 (identical to εBP) has been found to be present at a rather high concentration (over 100 μg/mL) in the cytosol of certain cells (Lindstedt et al., 1991). Since it is known that εBP is secreted by some cell types, it is reasonable to envision that this lectin is present at relatively high concentrations locally, under appropriate conditions.

It may be envisaged that εBP generated during inflammation to an initial stimulus may bind to mast cells at the inflammation site. These mast cells, loaded with εBP, may be activated as they attach to the extracellular matrix protein. In this way, εBP may contribute to the amplification of the inflammatory response. The effect of εBP is not necessarily restricted to mast cells. Different glycoprotein ligands for εBP are probably present on many other cell types. In fact, we have found binding of εBP to lymphocytes and endothelial cells. In the case of endothelial cells, εBP also binds to a restricted number of glycoproteins (unpublished). On the basis of the activity of εBP on mast cells described herein, it is reasonable to speculate that εBP can have a modulatory activity on various other cell types through binding to specific cell-surface glycoproteins. Possible extracellular functions of S-type lectins have been described previously (Barondes, 1984; Cooper & Barondes, 1990). The role of this type of lectin in modulating cellular activity by binding to critical cell-surface glycoproteins has also been proposed (Feizi & Childs, 1987). A picture appears to be emerging that εBP may be a broad-spectrum biological response modifier.

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